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7    **Title**

8    Cloning and characterization of Lxr and Srebp1, and their potential roles in regulation  
9    of LC-PUFA biosynthesis in rabbitfish *Siganus canaliculatus*

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21   **Keywords**

22   Elovl; Fad; LC-PUFA biosynthesis; Lxr; Rabbitfish *Siganus canaliculatus*; Srebp1

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## Title

Cloning and characterization of Lxr and Srebp1, and their potential roles in regulation of LC-PUFA biosynthesis in rabbitfish *Siganus canaliculatus*

## Abstract

Rabbitfish *Siganus canaliculatus* was the first marine teleost demonstrated to have the ability to biosynthesize C<sub>20-22</sub> long-chain polyunsaturated fatty acids (LC-PUFA) from C<sub>18</sub> PUFA precursors, which is generally absent or low in marine teleosts. Thus, understanding the molecular basis of LC-PUFA biosynthesis in rabbitfish will contribute to efforts aimed at optimizing LC-PUFA biosynthesis in teleosts, especially marine species. In the present study, the importance of the transcription factors liver X receptor (Lxr) and sterol regulatory element-binding protein 1 (Srebp1) in regulation of LC-PUFA biosynthesis in rabbitfish was investigated. First, full-length cDNAs of *Lxr* and *Srebp1* were cloned and characterized. The *Lxr* mRNA displayed a ubiquitous tissue expression pattern while *Srebp1* was highly expressed in eyes, brain and intestine. In rabbitfish primary hepatocytes treated with Lxr agonist T0901317, the expression of *Lxr* and *Srebp1* was activated, accompanied by elevated mRNA levels of  $\Delta 4$  and  $\Delta 6/\Delta 5$  fatty acyl desaturases (Fad), key enzymes of LC-PUFA biosynthesis, as well as peroxisome proliferator-activated receptor  $\gamma$  (Ppar $\gamma$ ). In addition, *Srebp1* displayed higher expression levels in liver of rabbitfish fed a vegetable oil diet or reared at 10 ppt salinity, which were conditions reported to increase the liver expression of  $\Delta 4$  and  $\Delta 6/\Delta 5$  *Fad* and LC-PUFA biosynthetic ability, than fish fed a

46 fish oil diet or reared at 32 ppt, respectively. These results suggested that Lxr and  
47 Srebp1 are involved in regulation of LC-PUFA biosynthesis probably by promoting  
48 the expression of two *Fads* in rabbitfish liver, which, to our knowledge, is the first  
49 report in marine teleosts.

50    **Abbreviations**

51	ARA	Arachidonic acid (20:4n-6)
52	cDNA	Complementary deoxyribonucleic acid
53	DHA	Docosahexaenoic acid (22:6n-3)
54	DMSO	Dimethyl Sulphoxide
55	Elovl	Elongases of very long-chain fatty acids
56	EFA	Essential fatty acids
57	EPA	Eicosapentaenoic acid (20:5n-3)
58	EtOH	Ethanol
59	Fad	Fatty acyl desaturases
60	FBS	Fetal bovine serum
61	FO	Fish oil
62	LC-PUFA	Long-chain polyunsaturated fatty acids
63	Lxr	Liver X receptor
64	NAMBS	Nan Ao Marine Biology Station
65	PCR	Polymerase chain reaction
66	Ppar	Peroxisome proliferator-activated receptors
67	PUFA	Polyunsaturated fatty acids
68	Srebp	Sterol regulatory element binding proteins
69	VO	Vegetable oil
70	22HC	22(R)-hydroxycholesterol

## Introduction

Long-chain ( $\geq C_{20}$ ) polyunsaturated fatty acids (LC-PUFA), such as eicosapentaenoic (20:5n-3, EPA) and docosahexaenoic (22:6n-3, DHA) acids, are essential fatty acids (EFA) for marine teleosts which, compared to freshwater and salmonid species, are historically regarded as species with limited capability for LC-PUFA biosynthesis from  $C_{18}$  PUFA precursors [1,2]. Hence in aquaculture, LC-PUFA-rich fish oil (FO) is utilized in feeds to satisfy the EFA requirement of marine teleosts [1]. However, FO is derived from wild capture fisheries that have reached their maximum sustainable level, and this has paradoxically constrained the development of marine aquaculture. Consequently, feasible and sustainable alternatives to FO are urgently required and vegetable oils (VO) have been the prime candidates [3]. In contrast to FO, VOs are rich in  $C_{18}$  PUFA but devoid of n-3 LC-PUFA and so replacement of dietary FO by VO results in reduced flesh n-3 LC-PUFA contents of farmed fish, which compromises their nutritional quality for humans [3]. Considerable attention therefore has focused on improving our understanding of the molecular basis for LC-PUFA biosynthesis, which will facilitate the efficient and effective utilization of sustainable plant lipid sources while maintaining the nutritional quality of farmed fish, especially marine teleosts.

In vertebrates, LC-PUFA biosynthesis initiates from  $C_{18}$  PUFA and requires a series of desaturation and elongation steps catalyzed by fatty acid desaturases (Fad) and elongases of very long-chain fatty acids (Elovl) [4,5]. In teleosts, all *Fad* genes cloned to date are homologous to mammalian *Fads2* and their enzyme products display

93  $\Delta 4$ ,  $\Delta 5$  or  $\Delta 6$  specificity, respectively, or act in a bifunctional manner [5].  $\Delta 4$  Fad  
94 from vertebrates was first identified in rabbitfish *Siganus canaliculatus* by our group  
95 [6], followed by Senegalese sole (*Solea senegalensis*) [7], Mexican whitefish  
96 (*Chirostoma estor*) [8] and striped snakehead (*Channa striata*) [9], and separate  $\Delta 5$   
97 Fad have been identified in Atlantic salmon (*Salmo salar*) [10] and rainbow trout  
98 (*Oncorhynchus mykiss*) [11]. Bifunctional  $\Delta 5/\Delta 6$  Fad were identified in zebrafish  
99 (*Danio rerio*) [12], rabbitfish [6] and Mexican whitefish [8], while  $\Delta 6$  Fad have been  
100 found in many fish species [5,13]. In addition, elongase genes in teleosts include  
101 *Elovl2*, *Elovl4* and *Elovl5*, among which *Elovl5* genes have been identified in most  
102 teleosts studied to date [5]. Based on the presence and substrate specificity of the key  
103 enzymes involved in LC-PUFA biosynthesis, studies increasingly focused on the  
104 promoter of *Fads* where response elements for transcription factors were identified,  
105 such as sterol regulatory element binding proteins (Srebp), nuclear factor Y (NF-Y)  
106 and specificity protein 1 (Sp1) [14-16]. However, the mechanisms of transcriptional  
107 regulation for LC-PUFA biosynthesis are largely unexplored, especially in marine  
108 teleosts.

109 The Srebp family includes three members, Srebp1a and 1c, and Srebp2 in  
110 mammals and, among them, Srebp1 plays a major role in regulation of fatty acid  
111 synthesis [17]. Furthermore, Srebp1c can be activated by Liver X receptor (Lxr), a  
112 class I nuclear receptor that can be activated by oxysterols derived from cholesterol  
113 oxidation [18-20]. In mouse liver, it was established that Lxr and Srebp1 formed a  
114 functional pathway (called “Lxr-Srebp1 pathway”) that stimulates the production of

PUFA by transcriptional activation of  $\Delta 5$  and  $\Delta 6$  desaturase (encoded by *Fads1* and *Fads2* genes, respectively) and Elovl5 [21]. In teleosts, the promoting effect of the Lxr-Srebp1 pathway on LC-PUFA biosynthesis has been implied in anadromous Atlantic salmon by increasing the expression of  $\Delta 5$  and  $\Delta 6$  *Fad* genes [16,22-24]. However, little is known about the presence or efficacy of Lxr-Srebp1 pathway in marine teleosts.

Besides Lxr and Srebp1, the peroxisome proliferator-activated receptors (Ppar) are also important regulators of lipid metabolism [25]. The Ppar family consists of three subtypes encoded by discrete genes [25]. In mammals, Ppar $\alpha$  and Ppar $\beta$  are activated by fatty acids or their derivatives, and play pleiotropic roles in lipid metabolism, i.e. stimulating the expression of genes related to peroxisomal and mitochondrial fatty acid oxidation, and LC-PUFA biosynthesis [26]. Ppar $\gamma$  has been associated with adipocyte differentiation and lipogenesis [27]. In mammals, Ppar $\alpha$  and Lxr interact in controlling *Srebp1* activation [28,29]. However, our understanding of the interaction between Ppar and Lxr in LC-PUFA biosynthesis in teleosts remains limited.

Rabbitfish *Siganus canaliculatus*, a true herbivore consuming algae and seagrass in nature, is widespread along the Indo-West Pacific coast and there is a commercial fishery. In aquaculture, rabbitfish can be domesticated to accept formulated feeds and, in recent years, rabbitfish farming has expanded rapidly in southeastern Asia, including China, due to its popularity in markets. It is noteworthy that *S. canaliculatus* was the first marine teleost demonstrated to have the ability of bioconverting C<sub>18</sub>

PUFA to LC-PUFA, and now all the key enzymes required for LC-PUFA biosynthesis have been characterized in this species, including  $\Delta 4$  Fad,  $\Delta 6/\Delta 5$  Fad, Elovl4 and Elovl5 [6,30,31]. Thus, rabbitfish provides a favourable model to investigate the regulatory mechanisms of LC-PUFA biosynthesis in teleosts. Besides, previous studies showed that dietary lipid source and ambient salinity influenced the LC-PUFA biosynthetic ability of rabbitfish as well as the liver expression of the key enzymes mentioned above [31,32], which suggested a repertoire of regulatory machinery responsible for liver LC-PUFA biosynthesis that is largely unexplored.

Accordingly, the present study aimed to investigate the potential role of Lxr and Srebp1 in the regulation of LC-PUFA biosynthesis in rabbitfish *S. canaliculatus*. First, the cDNAs encoding Lxr and Srebp1 were cloned and characterized, followed by determination of their tissue distribution. Subsequently, the expression levels of *Lxr* and *Srebp1* were measured in rabbitfish primary hepatocytes incubated with Lxr agonists, including T0901317, GW3965 and 22(R)-hydroxycholesterol (22HC), along with other genes related to LC-PUFA biosynthesis including  $\Delta 4$  Fad,  $\Delta 6/\Delta 5$  Fad, *Elovl5*, *Ppara*, *Ppar $\beta$*  and *Ppar $\gamma$* . Furthermore, the mRNA level of *Lxr* and *Srebp1* were evaluated in livers of rabbitfish reared at different ambient salinities with diets containing VO or FO. The results provide further insight into the regulatory mechanisms of LC-PUFA biosynthesis in rabbitfish and teleosts, and also provide a foundation to optimize the LC-PUFA biosynthetic pathway in teleosts, which could facilitate the efficient and effective utilization of sustainable vegetable lipid resources in marine aquaculture.



## **Materials and Methods**

### **Diets and feeding trials**

Two iso-proteic and iso-lipidic diets containing 35 % crude protein and 8 % crude lipid were formulated using either fish oil (rich in LC-PUFA) or a blend of canola oil and perilla oil (LC-PUFA-free) as lipid sources and named as FO and VO diets, respectively. Dietary compositions, experimental animal preparation and the feeding trial have all been described in detail previously, along with the detailed parameters on the growth performance and lipid composition of fish [32]. Briefly, juvenile rabbitfish (body mass approximately 13 g, sex indistinguishable visually) were caught off the coast near Nan Ao Marine Biology Station (NAMBS) of Shantou University, Southern China. Initially, 500 fish were maintained in an indoor seawater (32 ppt) pool for one month to adapt to the laboratory conditions. Subsequently, 250 fish were gradually acclimated to brackish water (10 ppt) for one month, followed by a further two weeks' period at 10 ppt while the other half remained in seawater throughout. The fish were then starved for 24 h, anesthetized with 0.01 % 2-phenoxyethanol (Sigma-Aldrich, St. Louis, MO, USA), individually weighed and 20 fish each allocated randomly to 12 cylindrical tanks (90 cm diameter, 100 cm depth) with triplicate tanks for each diet (FO and VO) at both salinities (10 and 32 ppt). The feeding trial lasted for 8-weeks in an indoor aquarium system at NAMBS. During the trial, oxygen-saturation was maintained by aeration, temperature was kept at  $22 \pm 3$  °C and photoperiod was set at a 12 h light: 12 h dark cycle.

180

## 181 **Tissue collection**

182 The liver of one fish in seawater during the initial acclimation period was collected  
183 for RNA isolation followed by cDNA cloning of *Lxr* and *Srebp1*. In addition, tissues  
184 including heart, liver, spleen, gill, muscle, eyes, intestine and brain were sampled  
185 from a further three individuals for determination of tissue distribution of *Lxr* and  
186 *Srebp1* mRNA. To determine the effects of diet and salinity on gene expression, livers  
187 were collected from three fish in seawater at the beginning of the feeding trial as  
188 initial control samples, and at the end of the trial, livers were collected from two fish  
189 per tank (six fish per dietary treatment per salinity). Tissue samples were immediately  
190 frozen in liquid nitrogen and subsequently stored at -80 °C before analysis. All  
191 procedures performed on fish conformed with the National Institutes of Health guide  
192 for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978)  
193 and approved by the Animal Care and Use Committee of Shantou University  
194 (Guangdong, China). All surgery was performed under 0.01 % 2-phenoxyethanol  
195 anesthesia, and all efforts were made to minimize suffering of fish.

196

## 197 **Rabbitfish primary hepatocyte isolation and incubation with Lxr agonists**

198 Rabbitfish were fasted 24 h and then euthanized with 0.01 % 2-phenoxyethanol,  
199 followed by immersion in 70 % ethanol to sterilize the external surfaces. Primary  
200 hepatocytes were prepared from a pool of three livers as described previously [33].

Briefly, chopped livers were digested by 0.1 % collagenase/0.25 % hyaluronidase (Sigma-Aldrich, St. Louis, USA) followed by filtering through a 100 µm strainer. Isolated cells (viability  $\geq$  98 % evaluated by Trypan Blue dye exclusion) were seeded in 6-well plates at a density of  $2 \times 10^6$  cells per well with 2 ml DMEM/F12 medium containing 20 % fetal bovine serum (FBS), 100 U/ml penicillin and 100 µg/ml streptomycin, followed by incubation at 24 °C/4 % CO<sub>2</sub> for 24 h. The hepatocytes were incubated in FBS-free DMEM/F12 medium for 3 h prior to incubation with the following Lxr agonists: synthetic ligands T0901317 (1 and 2 µM) and GW3965 (2 and 4 µM), natural ligand 22(R)-hydroxycholesterol (22HC 5 and 10 µM), and treatment vehicles (DMSO for T091317 and GW3965, and ethanol (EtOH) for 22HC) on the basis of previous studies [34-36]. In addition, incubations without chemical supplement were included as control. After 6 h incubation, cells were lysed in wells and harvested for subsequent RNA extraction.

#### **RNA isolation and cDNA synthesis**

Total RNA from rabbitfish tissues was extracted by Trizol reagent (Invitrogen, Carlsbad, CA, USA) and total RNA from rabbitfish primary hepatocytes was isolated and purified by RNAPrep pure cell/bacteria kit (Tiangen Biotech, Beijing, China). The concentration and quality of total RNA preparations were confirmed by spectrophotometry (NanoDrop 2000, Thermo Scientific, USA) and agarose gel electrophoresis. For cloning partial sequences, one µg total RNA from liver was

subjected to cDNA synthesis using the Cloned AMV First-Strand cDNA Synthesis Kit (Invitrogen, USA). For semi-quantitative or quantitative analysis of target gene expression level, cDNA was synthesized from 1 µg or 500 ng of total RNA from rabbitfish tissues or primary hepatocytes, respectively, using FastQuant RT Kit (with gDNase) (Tiangen Biotech, Beijing, China).

### **Molecular cloning of rabbitfish *Lxr* and *Srebp1* cDNAs**

Partial cDNA fragments of *Lxr* and *Srebp1* were obtained by polymerase chain reaction (PCR) using primer pair *lxr*-partial-F/R and *srebp1*-partial-F/R, respectively. Primers were designed on highly conserved regions of cDNA sequences based on alignments of human (*Homo sapiens*; NCBI accession: *Lxra*, AB307698.1; *Srebp1*, BC063281.1), mouse (*Mus musculus*; *Lxra*, AJ132601.1; *Srebp1*, BC056922.1), chicken (*Gallus gallus*; *Lxr*, AF492498.1; *Srebp1*, NM\_204126.2), Atlantic salmon (*Lxr*, FJ470290.1; *Srebp1*, HM561860.1) and zebrafish (*Lxr*, BC092160.1; *Srebp1*, NM\_001105129.1). The PCR was performed with initial denaturation at 95 °C for 5 min and 35 cycles of 94 °C for 30 s, 60 °C for 30 s, 72 °C for 1 min, followed by a final extension at 72 °C for 10 min.

The full-length cDNA sequences of *Lxr* and *Srebp1* were cloned by 3' and 5' rapid amplification of cDNA ends (RACE) PCR based on partial cDNA fragments using the GeneRacer<sup>®</sup> kit (Invitrogen, USA). According to the manufacturer's instructions, specific primers *lxr*-5'-F1/F2 and *srebp1*-5'-F1/F2 were designed to

243 amplify the 5' ends of *Lxr* and *Srebp1* cDNAs while *lxr*-3'-R1/R2 and  
244 *srebp1*-3'-R1/R2 were prepared for the 3' ends of the two cDNAs, respectively. The  
245 multiple amplicons from PCR and RACE were assembled and the full-length cDNA  
246 sequences of rabbitfish *Lxr* and *Srebp1* were further generated by "end to end" PCR.  
247 All primer sequences are shown in Table 1.

248

#### 249 **Sequence and phylogenetic analysis**

250 ClustalW2 (<http://www.ebi.ac.uk/Tools/msa/clustalw2/>) was used to align the  
251 deduced amino acid (aa) sequences of the newly cloned rabbitfish *Lxr* and *Srebp1*  
252 cDNA with their corresponding orthologs from other species, including human (*Lxr* $\alpha$ ,  
253 NCBI accession: AAA85856.1), mouse (*Lxr* $\alpha$ , CAB51923.1), chicken (*Lxr* $\alpha$ ,  
254 NP\_989873.1; *Srebp1*, NP\_989457.2), Atlantic salmon (*Lxr* $\alpha$ , NP\_001138893.1;  
255 *Srebp1*, ADN28371.1) and zebrafish (*Lxr* $\alpha$ , NP\_001017545.1; *Srebp1*,  
256 NP\_001098599.1). The aa sequences for *Srebp1* isoforms of human (*Srebp1a*,  
257 P36956-1; *Srebp1c*, P36956-3) and mouse (*Srebp1a*, Q9WTN3-1; *Srebp1c*,  
258 Q9WTN3-3) were acquired from the UniProtKB database.

259 Mega5 [37] and the neighbor joining method were used to construct phylogenetic  
260 trees based on the aa sequences of *Lxr* and *Srebp* proteins from rabbitfish and other  
261 species including human, mouse, chicken, zebrafish, Atlantic salmon, carp (*Cyprinus*  
262 *carpio*), Japanese flounder (*Paralichthys olivaceus*), large yellow croaker  
263 (*Larimichthys crocea*), rainbow trout, tongue sole (*Cynoglossus semilaevis*), grass

264 carp (*Ctenopharyngodon idella*) and Japanese sea bass (*Lateolabrax japonicus*).

265

### 266 **Semi-quantitative PCR and Real-time qPCR assays**

267 Semi-quantitative PCR was conducted on tissue cDNA samples from three rabbitfish  
268 to determine the tissue distribution of *Lxr* and *Srebp1* mRNA, with *18S rRNA*  
269 (AB276993) as an internal control to check the efficiency of cDNA synthesis and  
270 cDNA integrity. To confirm the absence of genomic DNA contamination,  
271 non-template controls (NTC), consisting of reactions without reverse transcriptase,  
272 were also run. Semi-quantitative PCR was carried out with an initial denaturing step  
273 at 94 °C for 5 min, followed by 28 cycles of denaturation at 94 °C for 30 s, annealing  
274 at 60 °C for 30 s, extension at 72 °C for 30 s, and a final extension at 72 °C for 5 min.  
275 The primer pairs used for semi-quantitative PCR were the same as used for qPCR  
276 (Table 1).

277 The expression level of *Lxr*, *Srebp1*,  $\Delta 4$  *Fad* (GU594278.1),  $\Delta 6/\Delta 5$  *Fad*  
278 (EF424276.2), *Elovl5* (GU597350.1), *Ppara* (JF502070.1), *Ppar $\beta$*  (JF502071.1) and  
279 *Ppar $\gamma$*  (JF502072.1) mRNA was determined by qPCR on a LightCycler® 480  
280 thermocycler (Roche, Germany). All reactions were run in a total volume of 20  $\mu$ l  
281 containing 10  $\mu$ l LightCycler® 480 SYBR Green I Master (Roche, Germany), 0.4  $\mu$ M  
282 each of gene specific primer pairs (Table 1), 20 ng cDNA templates (10 ng/ $\mu$ l) and  
283 ddH<sub>2</sub>O in a white 96-well plate. The qPCR programs consisted of initial DNA  
284 denaturation at 94 °C for 5 min and 45 cycles of 95 °C for 10 s, 60 °C for 20 s, 72 °C

20 s, followed by a melting curve to confirm the amplification of a single product in each reaction. The amplification efficiency of primers was also determined by serial dilution of cDNA template in ddH<sub>2</sub>O. The relative RNA level of genes in each sample was normalized to *18S rRNA* expression and calculated by the comparative threshold cycle (Ct) method as fold change relative to initial control samples [38]. Triplicates of each reaction were conducted for each sample.

### **Statistical analysis**

All data were presented as means  $\pm$  SEM (n = 6 individuals for gene expression in liver of rabbitfish fed VO or FO diets at 10 ppt or 32 ppt salinity; n = 3 technical replicates for cell studies). In cell studies, differences among groups were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test at a significance of  $P < 0.05$ . A two-way ANOVA was used to analyze the differences of gene expression in rabbitfish liver, using FO and VO diets as the first independent variable, and the two salinity treatments (32 ppt and 10 ppt) as the second independent variable, and significant levels were considered at  $P < 0.05$ . All statistical analyses were performed using OriginPro 7.5 software (OriginLab Corporation, USA).

## Results

### Sequence characteristics of rabbitfish *Lxr* and *Srebp1* cDNAs

The full-length rabbitfish *Lxr* cDNA was 3055 bp containing a 5' untranslated region (UTR) of 407 bp, a 3' UTR of 1259 bp and an open reading frame (ORF) of 1389 bp encoding a protein of 462 aa. The nucleotide and deduced aa sequences were submitted to NCBI with accession numbers JF502074.1 and AFH35110.1, respectively. Rabbitfish *Lxr* has a typical structure of *Lxr* family proteins, including a DNA binding domain (DBD), ligand binding domain (LBD), activation function domains (AF-1/2) and a D (hinge) region (Fig. 1a). The full-length of *Srebp1* cDNA was 3952 bp containing 5' and 3' UTRs of 201 bp and 239 bp, respectively, and an ORF of 3513 bp encoding a 1171 aa protein. The nucleotide and aa sequences were submitted to NCBI with accession numbers JF502069.1 and AFH35105.1, respectively. The deduced rabbitfish *Srebp1* protein showed the conserved basic helix-loop-helix (bHLH) domain, which is essential for DNA binding as transcription factors (Fig. 1b and supplementary Fig. S1).

### Multiple sequence alignment and phylogenetic analysis

Alignment analysis of the deduced rabbitfish proteins with their orthologs in other species showed that rabbitfish *Lxr* shared high sequence identity with zebrafish (92 %) and salmon (85 %) and 74 -77 % identity with *Lxr $\alpha$*  from human and mouse, and *Lxr* from chicken (Fig. 1a). Similarly, phylogenetic analysis revealed that the *Lxr* proteins



of fish species were all clustered with mammalian Lxr $\alpha$  (Fig. 2a).

As for Srebp1, results showed that the rabbitfish Srebp1 shared 77 % and 72 % identity with those of Atlantic salmon and zebrafish, respectively, and 54 - 56 % identity with Srebp1a from human, mouse and chicken, and 54 % identity with Srebp1c from human and mouse (Fig. 1b). The N-terminal protein sequence alignment showed that the rabbitfish Srebp1 aa sequence was more similar to mammalian Srebp1a isoform (Fig. 1b). Phylogenetic analysis showed rabbitfish Srebp1 clustered with vertebrate Srebp1 rather than Srebp2, and closest to Srebp1 of *L. japonicus* (Fig. 2b).

#### **Tissue distribution of *Lxr* and *Srebp1* mRNA in rabbitfish**

The abundance of *Lxr* and *Srebp1* mRNA in different tissues was analyzed. Rabbitfish *Lxr* mRNA was detected in all studied tissues with lowest abundance in muscle and relatively high level in spleen and heart, and then tissues regarded as having high LC-PUFA biosynthetic ability including liver, intestine, eyes and brain (Fig. 3). Relatively high expression level of *Srebp1* was observed in eyes, intestine and brain, followed by heart, spleen, gill, and liver whereas the abundance of *Srebp1* mRNA in muscle was too low to be detected (Fig. 3).

#### **Effects of Lxr agonists on the expression of *Lxr*, *Srebp1* and other genes related to LC-PUFA biosynthesis in rabbitfish primary hepatocytes**

First, three Lxr agonists were tested for their effects on activating the expression of *Lxr* and *Srebp1* in rabbitfish hepatocytes. Results showed that the expression level of *Lxr* and *Srebp1* mRNA was increased 1.2 to 1.4-fold and ~9-fold, respectively, by T0901317, whereas 22HC promoted the expression of *Lxr* (~1.3-1.4 fold) and GW3965 promoted the expression of *Srebp1* (~6-13 fold), separately (Fig. 4). Based on minimum activating doses, T0901317 was determined as the most potent activator for *Lxr* and *Srebp1* expression in rabbitfish liver. Subsequently, the expression level of other genes involved in LC-PUFA biosynthesis was determined in hepatocytes incubated with T0901317. Results showed that  $\Delta 4$  *Fad* was induced by 1  $\mu$ M T0901317 (~1.7-fold), whereas 2  $\mu$ M T0901317 activated the transcription of both  $\Delta 4$  (~1.7-fold) and  $\Delta 6/\Delta 5$  *Fad* (~2-fold), but decreased the expression level of *Elovl5* as compared to 1  $\mu$ M T0901317 (Fig. 5). As for Ppar subtypes, T0901317 treatment increased the expression of *Ppar $\gamma$*  at 1  $\mu$ M, with no significant effect on the expression level of *Ppara $\alpha$*  and  $\beta$  (Fig. 5).

#### **Effects of dietary PUFA and ambient salinity on the expression of *Lxr* and *Srebp1* in rabbitfish liver**

The expression pattern of *Lxr* and *Srebp1* in rabbitfish liver was different in response to dietary lipid source and ambient salinity (Fig. 6). Rabbitfish fed FO diets displayed higher expression level of *Lxr* than fish fed VO diets ( $P < 0.05$ ), while ambient salinity produced no significant change on the expression of *Lxr* in rabbitfish liver. In addition,

367 the expression of *Srebp1* was higher in the liver of rabbitfish fed VO diets or reared at  
368 10 ppt than that of fish fed FO diets or reared at 32 ppt ( $P < 0.05$ ). There was no  
369 significant interaction between dietary lipid source and ambient salinity on the  
370 expression of *Lxr* or *Srebp1* in rabbitfish liver.  
371

## Discussion

### Comparison of *Lxr* and *Srebp1* sequences between rabbitfish and other species

As a class I nuclear receptor, *Lxr* possesses two important functional regions, LBD and DBD, that are associated with ligand binding and downstream target interaction [39]. Herein, the LBD and DBD of rabbitfish *Lxr* showed high identity with those of *Lxr* orthologs from other species, which indicated similar roles in ligand recognition and downstream target activation. In mammals, there are two isoforms of *Lxr*, *Lxr* $\alpha$  and *Lxr* $\beta$ , with overlapping or distinct functions [40]. However, rabbitfish possesses a unique form of *Lxr* that clustered with mammalian *Lxr* $\alpha$ , and were *Lxr* orthologs of other fish species in the phylogenetic analysis. This suggested the gene loss of *Lxr* $\beta$  in teleosts, which was consistent with observations in zebrafish, rainbow trout and salmon [34,41].

The *Srebps* subfamily is encoded by two distinct genes, designated *Srebp1* and *Srebp2*, in vertebrates [17,23], and the cDNA characterized in the present study was homologous to mammalian *Srebp1*. In mammals, the transcription products of *Srebp1* consist of two isoforms, *Srebp1a* and *1c*, which differ only in the first exon as a result of alternative splicing [42]. However, this is not the case in rabbitfish, although it cannot be excluded that the cDNA used for cloning was synthesized from liver RNA. Specifically, the rabbitfish *Srebp1* was closely related to mammal *Srebp1a* rather than *Srebp1c*, which was also observed in zebrafish, salmon and Japanese sea bass [23,43]. In mammals, the relatively long N-terminal AF-1 domain confers the *Srebp1a* with

potent transactivation activity while Srebp1c with a short AF-1 domain requires further post-translational modification to achieve complete activity [44,45]. Thus, the high similarity of rabbitfish Srebp1 to mammal Srebp1a ensures its efficacy in transactivation.

### **Tissue distribution of *Lxr* and *Srebp1* mRNA implies their relevance in LC-PUFA biosynthesis in rabbitfish**

The tissue expression pattern of *Lxr* varies among species [39,41,46]. In the present study, rabbitfish *Lxr* displayed high expression level in spleen, which is consistent with the observation in rainbow trout, but low in muscle, similar to the finding in salmon [41]. In yellow catfish (*Pelteobagrus fulvidraco*), transcript variants of *Lxr $\alpha$*  (*Lxr $\alpha$ -1* and *Lxr $\alpha$ -2*) were identified with different tissue expression patterns [46]. Thus, the varied tissue distribution profile of *Lxr* mRNA among fish species may be due to the presence of multiple *Lxr* isoforms. In addition, the ubiquitous expression pattern of *Lxr* in rabbitfish tissues implied its pleiotropic effects in physiology.

Srebp1 play major role in the regulation of fatty acid synthesis in mammals [17]. In the present study, *Srebp1* mRNA displayed high abundance in eyes, brain and intestine, which are tissues with high level of  $\Delta 4$  and  $\Delta 6$  *Fad* mRNA and likewise potent LC-PUFA biosynthetic activity [31,33]. Thus, Srebp1 might be implicated in the regulation of LC-PUFA biosynthesis in rabbitfish. However, *Srebp1* mRNA is lacking in rabbitfish muscle, which can be attributed to the reduced expression of *Lxr*

that indirectly regulates fatty acid synthesis by driving *Srebp1* transcription in mammals [20,21].

#### **Potential role and mechanism for Lxr and Srebp1 in regulation of LC-PUFA biosynthesis in rabbitfish liver**

As a ligand-activated nuclear receptor, Lxr can be activated by synthetic or natural agonists [18,39,47]. In the present study, agonists increased the expression of *Lxr* in rabbitfish hepatocytes, which can be explained by the positive autoregulation of Lxr that was shown to have LXREs in its own promoter in human and mice [48,49]. A similar autoregulatory situation has also been observed in recent studies performed in rainbow trout and Atlantic salmon [22,35,36]. *Srebp1* was established as a target gene for Lxr in mammal and rodent systems [19,20]. Consistent with this, the expression of *Srebp1* was increased by the Lxr agonists T0901317 and GW3965, whereas the 22HC produced no significant effects. In human myotubes, differential effects of T0901317 and 22HC were observed in regulation of genes involved in lipid metabolism, although both of them are effective agonists of Lxr $\alpha$  [50]. T0901317 and GW3965 were more effective activators for the Lxr of Atlantic salmon than 22HC [22]. Apparently, the effectiveness of Lxr agonists were model-specific, and thus in the present study, T0901317 was regarded as a potent activator for the expression of both Lxr and *Srebp1* with maximal effect at minimum dose, which also indicated the conservation of Lxr-*Srebp1* pathway in rabbitfish.

Fad and Elovl are key enzymes involved in LC-PUFA biosynthesis, and their gene expressions are commonly correlated to the LC-PUFA biosynthetic ability [4,5]. In mouse liver, the Lxr-Srebp1 pathway was demonstrated to be involved in LC-PUFA biosynthetic regulation through activating the expression of  $\Delta 5$ ,  $\Delta 6$  desaturase and Elovl5 [21]. In Atlantic salmon SHK-1 cells, T0901317 promoted the expression of  $\Delta 5$  and  $\Delta 6$  *Fad* but not *Elovl5*, accompanied by the increased expression of *Lxr* and *Srebp1* [22]. As such, in the present study, the increased expression of  $\Delta 4$  and  $\Delta 6/\Delta 5$  *Fad* in rabbitfish hepatocytes incubated with T0901317 might be a result of activated Lxr-Srebp1 pathway, where *Elovl5* might not be included.

Interestingly, the transcription of the  $\Delta 4$  *Fad* was more sensitive to the activation of Lxr and Srebp1 than  $\Delta 6/\Delta 5$  *Fad* in the present study. As the first vertebrate reported with  $\Delta 4$  desaturation activity and the first marine teleost with  $\Delta 6/\Delta 5$  desaturation activity, rabbitfish possesses two mechanisms for DHA biosynthesis from EPA. The first mechanism is the so-called “Sprecher pathway”, where EPA undergoes two successive elongation steps to produce 24:5n-3, followed by a second  $\Delta 6$  desaturation step to produce 24:6n-3 that is chain-shortened to DHA by partial  $\beta$ -oxidation [51]. The second mechanism is the “ $\Delta 4$  desaturation pathway” that requires a single elongation to 22:5n-3, which is then directly  $\Delta 4$  desaturated to generate DHA [6]. Our recent study showed that there is coordination between the two pathways, in which miR-17 is involved through suppressing  $\Delta 4$  *Fad*

post-transcriptionally rather than  $\Delta 6$  *Fad* [33]. Likewise, the results presented herein further suggested the coordinating machinery at transcriptional level, possibly due to the distinct promoter structures between the two *Fads*. In addition, DHA is difficult to oxidize since its  $\Delta 4$  bond must be removed by peroxisomal  $\beta$ -oxidation, as compared to EPA [52]. Herein, the higher sensitivity of  $\Delta 4$  *Fad* towards the Lxr and Srebp1 activation may facilitate the bioconversion of EPA into DHA in rabbitfish liver.

Dietary PUFA and ambient salinity were demonstrated as important factors that influence LC-PUFA biosynthesis and *Fad* expression in rabbitfish liver [31,32], which provided the physiological context to further determine the role of Lxr and Srebp1 in the regulation of LC-PUFA biosynthesis. Results showed that the liver expression pattern of *Srebp1* was correlated with that of  $\Delta 4$  and  $\Delta 6/\Delta 5$  *Fad* reported previously, with higher mRNA level in liver of rabbitfish fed VO diets or reared at 10 ppt than that of fish fed FO diets or reared at 32 ppt [31,32]. In combination with the *in vitro* results, there is good reason to speculate that Srebp1 is a critical transcription factor for the expression of  $\Delta 4$  and  $\Delta 6/\Delta 5$  *Fad* in rabbitfish liver. In contrast to *Srebp1*, *Lxr* displayed higher expression level in liver of rabbitfish fed FO diets than that of fish fed VO diets, while ambient salinity produced no significant effects on the expression of *Lxr*. Similarly, the asynchronous expression of *Lxr* and *Srebp1* in circadian rhythmicity has been observed in the liver of Atlantic salmon [24]. As such, Lxr was not the only regulator for *Srebp1* expression in rabbitfish liver physiologically and the



476 extent to which Lxr impacts on LC-PUFA biosynthesis in rabbitfish liver should be  
477 further investigated.

478 In mammals, LC-PUFA inhibited the transcription of *Srebp1c* [53], accelerated  
479 *Srebp1* mRNA decay [54] and suppressed proteolytic release of Srebp1 protein [55].  
480 Similarly, the mRNA level of *Srebp1* was lower in the liver of sea bass fed FO  
481 (LC-PUFA-rich) diets than that of fish fed VO diets containing low LC-PUFA level  
482 [56]. In addition, FO is also rich in cholesterol, the precursor of oxysterols that are  
483 endogenous ligands for Lxr, which is absent in VO [3]. Thus in the present study, the  
484 different expression pattern of *Lxr* and *Srebp1* in the liver of rabbitfish fed VO or FO  
485 diets can be explained by their specific responses to dietary nutrients such as  
486 LC-PUFA and cholesterol. Furthermore, these results suggested the complexity of the  
487 molecular mechanisms of transcriptional regulation of *Lxr* and *Srebp1* in LC-PUFA  
488 biosynthesis of teleosts, which requires further investigation.

489 Ppar interact with Lxr in regulation of lipid metabolism in a tissue- and  
490 species-dependent manner [22,35,36,57]. In the present study, only the expression of  
491 *Ppar $\gamma$*  was up-regulated by Lxr activation. In rodent adipocytes, Lxr $\alpha$  activated the  
492 transcription of *Ppar $\gamma$*  and induced adipogenesis [58], but this was not the case for  
493 *Ppar $\gamma$*  in rainbow trout adipocytes [36]. In SHK cells of Atlantic salmon, the  
494 activation of Lxr did not affect the expression of any of the *Ppar* subtypes [22]. Hence,  
495 the mechanism for the promotion of *Ppar $\gamma$*  by Lxr and its significance in LC-PUFA  
496 biosynthesis should be further investigated.

In the present study, the cDNAs encoding *Lxr* and *Srebp1* were cloned and characterized from rabbitfish *S. canaliculatus*. Furthermore, our results implied their roles in LC-PUFA biosynthesis, probably by activating the expression of  $\Delta 4$  and  $\Delta 6/\Delta 5$  *Fad* in liver. In return, the expression of *Lxr* and *Srebp1* in liver was also affected by dietary lipid sources. Additionally, *Ppar $\gamma$*  was inferred as a possible target for Lxr although the mechanism and significance remained to be clarified. These results increased our understanding of the regulatory mechanisms for LC-PUFA biosynthesis in rabbitfish and teleosts, which will contribute to optimizing the LC-PUFA biosynthetic pathway in marine teleosts and developing more efficient, low-cost aquaculture diets based on vegetable lipid sources.

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## **Conflict of Interest**

The authors have declared that no competing interests exist.

## 517    **References**

- 518        1. Tocher DR (2010) Fatty acid requirements in ontogeny of marine and freshwater fish. *Aquac*  
519            Res 41(5):717-732
- 520        2. Tocher DR, Agaba M, Hastings N, Teale AJ (2003) Biochemical and molecular studies of the  
521            fatty acid desaturation pathway in fish. Paper presented at the The Big Fish Bang, Browman, H.,  
522            Skiftesvik, A.B.
- 523        3. Turchini GM, Torstensen BE, Ng W (2009) Fish oil replacement in finfish nutrition. *Rev*  
524            *Aquacult* 1(1):10-57
- 525        4. Cook HW, McMaster CR (2004) In: Vance DE, Vance JE (eds) Fatty acid desaturation and  
526            chain elongation in eukaryotes, 4th edn. Elsevier, Amsterdam
- 527        5. Castro LF, Tocher DR, Monroig O (2016) Long-chain polyunsaturated fatty acid biosynthesis in  
528            chordates: Insights into the evolution of Fads and Elovl gene repertoire. *Prog Lipid Res*  
529            62:25-40
- 530        6. Li Y, Monroig O, Zhang L, Wang S, Zheng X, Dick JR, You C, Tocher DR (2010) Vertebrate  
531            fatty acyl desaturase with  $\Delta 4$  activity. *Proc Natl Acad Sci USA* 107(39):16840-16845
- 532        7. Morais S, Castanheira F, Martinez-Rubio L, Conceicao LE, Tocher DR (2012) Long chain  
533            polyunsaturated fatty acid synthesis in a marine vertebrate: ontogenetic and nutritional  
534            regulation of a fatty acyl desaturase with  $\Delta 4$  activity. *Biochim Biophys Acta* 1821(4):660-671
- 535        8. Fonseca-Madrigal J, Navarro JC, Hontoria F, Tocher DR, Martinez-Palacios CA, Monroig O  
536            (2014) Diversification of substrate specificities in teleostei Fads2: characterization of  $\Delta 4$  and  
537             $\Delta 6/\Delta 5$  desaturases of *Chirostoma estor*. *J Lipid Res* 55(7):1408-1419
- 538        9. Kuah MK, Jaya-Ram A, Shu-Chien AC (2015) The capacity for long-chain polyunsaturated

539 fatty acid synthesis in a carnivorous vertebrate: Functional characterisation and nutritional  
540 regulation of a Fads2 fatty acyl desaturase with  $\Delta 4$  activity and an Elovl5 elongase in striped  
541 snakehead (*Channa striata*). Biochim Biophys Acta 1851(3):248-260

542 10. Hastings N, Agaba MK, Tocher DR, Zheng X, Dickson CA, Dick JR, Teale AJ (2004)  
543 Molecular cloning and functional characterization of fatty acyl desaturase and elongase cDNAs  
544 involved in the production of eicosapentaenoic and docosahexaenoic acids from  $\alpha$ -linolenic acid  
545 in Atlantic salmon (*Salmo salar*). Mar Biotechnol 6(5):463-474

546 11. Abdul HN, Carmona-Antonanzas G, Monroig O, Tocher DR, Turchini GM, Donald JA (2016)  
547 Isolation and Functional Characterisation of a fads2 in Rainbow Trout (*Oncorhynchus mykiss*)  
548 with  $\Delta 5$  Desaturase Activity. PLoS One 11(3):e150770

549 12. Hastings N, Agaba M, Tocher DR, Leaver MJ, Dick JR, Sargent JR, Teale AJ (2001) A  
550 vertebrate fatty acid desaturase with  $\Delta 5$  and  $\Delta 6$  activities. Proc Natl Acad Sci USA  
551 98(25):14304-14309

552 13. Vagner M, Santigosa E (2011) Characterization and modulation of gene expression and  
553 enzymatic activity of delta-6 desaturase in teleosts: A review. Aquaculture 315(1-2):131-143

554 14. Xu H, Dong X, Ai Q, Mai K, Xu W, Zhang Y, Zuo R (2014) Regulation of tissue LC-PUFA  
555 contents,  $\Delta 6$  fatty acyl desaturase (FADS2) gene expression and the methylation of the putative  
556 FADS2 gene promoter by different dietary fatty acid profiles in Japanese seabass (*Lateolabrax*  
557 *japonicus*). PLoS One 9(1):e87726

558 15. Geay F, Zambonino-Infante J, Reinhardt R, Kuhl H, Santigosa E, Cahu C, Mazurais D (2012)  
559 Characteristics of *fads2* gene expression and putative promoter in European sea bass  
560 (*Dicentrarchus labrax*): comparison with salmonid species and analysis of CpG methylation.

561 Mar Genomics 5:7-13

562 16. Zheng X, Leaver MJ, Tocher DR (2009) Long-chain polyunsaturated fatty acid synthesis in fish:

563 Comparative analysis of Atlantic salmon (*Salmo salar* L.) and Atlantic cod (*Gadus morhua* L.)

564  $\Delta 6$  fatty acyl desaturase gene promoters. Comp Biochem Physiol B Biochem Mol Biol

565 154(3):255-263

566 17. Horton JD, Goldstein JL, Brown MS (2002) SREBPs: activators of the complete program of

567 cholesterol and fatty acid synthesis in the liver. J Clin Invest 109(9):1125-1131

568 18. Aranda A, Pascual A (2001) Nuclear hormone receptors and gene expression. Physiol Rev

569 81(3):1269-1304

570 19. Yoshikawa T, Shimano H, Amemiya-Kudo M, Yahagi N, Hasty AH, Matsuzaka T, Okazaki H,

571 Tamura Y, Iizuka Y, Ohashi K, Osuga J, Harada K, Gotoda T, Kimura S, Ishibashi S, Yamada

572 N (2001) Identification of liver X receptor-retinoid X receptor as an activator of the sterol

573 regulatory element-binding protein 1c gene promoter. Mol Cell Biol 21(9):2991-3000

574 20. Repa JJ, Liang G, Ou J, Bashmakov Y, Lobaccaro JM, Shimomura I, Shan B, Brown MS,

575 Goldstein JL, Mangelsdorf DJ (2000) Regulation of mouse sterol regulatory element-binding

576 protein-1c gene (SREBP-1c) by oxysterol receptors, LXR $\alpha$  and LXR $\beta$ . Genes Dev

577 14(22):2819-2830

578 21. Qin Y, Dalen KT, Gustafsson JA, Nebb HI (2009) Regulation of hepatic fatty acid elongase 5

579 by LXR $\alpha$ -SREBP-1c. Biochim Biophys Acta 1791(2):140-147

580 22. Carmona-Antonanzas G, Tocher DR, Martinez-Rubio L, Leaver MJ (2014) Conservation of

581 lipid metabolic gene transcriptional regulatory networks in fish and mammals. Gene 534(1):1-9

582 23. Minghetti M, Leaver MJ, Tocher DR (2011) Transcriptional control mechanisms of genes of

lipid and fatty acid metabolism in the Atlantic salmon (*Salmo salar* L.) established cell line, SHK-1. *Biochim Biophys Acta* 1811(3):194-202

24. Betancor MB, McStay E, Minghetti M, Migaud H, Tocher DR, Davie A (2014) Daily rhythms in expression of genes of hepatic lipid metabolism in Atlantic salmon (*Salmo salar* L.). *PLoS One* 9(9):e106739

25. Jump DB, Tripathy S, Depner CM (2013) Fatty acid-regulated transcription factors in the liver. *Annu Rev Nutr* 33:249-269

26. Desvergne B, Michalik L, Wahli W (2006) Transcriptional regulation of metabolism. *Physiol Rev* 86(2):465-514

27. Tontonoz P, Spiegelman BM (2008) Fat and beyond: the diverse biology of PPAR $\gamma$ . *Annu Rev Biochem* 77:289-312

28. Yoshikawa T, Ide T, Shimano H, Yahagi N, Amemiya-Kudo M, Matsuzaka T, Yatoh S, Kitamine T, Okazaki H, Tamura Y, Sekiya M, Takahashi A, Hasty AH, Sato R, Sone H, Osuga J, Ishibashi S, Yamada N (2003) Cross-talk between peroxisome proliferator-activated receptor (PPAR)  $\alpha$  and liver X receptor (LXR) in nutritional regulation of fatty acid metabolism. I. PPARs suppress sterol regulatory element binding protein-1c promoter through inhibition of LXR signaling. *Mol Endocrinol* 17(7):1240-1254

29. Ide T, Shimano H, Yoshikawa T, Yahagi N, Amemiya-Kudo M, Matsuzaka T, Nakakuki M, Yatoh S, Iizuka Y, Tomita S, Ohashi K, Takahashi A, Sone H, Gotoda T, Osuga J, Ishibashi S, Yamada N (2003) Cross-talk between peroxisome proliferator-activated receptor (PPAR)  $\alpha$  and liver X receptor (LXR) in nutritional regulation of fatty acid metabolism. II. LXRs suppress lipid degradation gene promoters through inhibition of PPAR signaling. *Mol Endocrinol*

605 17(7):1255-1267

- 606 30. Monroig O, Wang S, Zhang L, You C, Tocher DR, Li Y (2012) Elongation of long-chain fatty  
607 acids in rabbitfish *Siganus canaliculatus*: Cloning, functional characterisation and tissue  
608 distribution of Elovl5- and Elovl4-like elongases. *Aquaculture* 350:63-70
- 609 31. Li Y, Hu C, Zheng Y, Xia X, Xu W, Wang S, Chen W, Sun Z, Huang J (2008) The effects of  
610 dietary fatty acids on liver fatty acid composition and  $\Delta^6$ -desaturase expression differ with  
611 ambient salinities in *Siganus canaliculatus*. *Comp Biochem Physiol B Biochem Mol Biol*  
612 151(2):183-190
- 613 32. Xie D, Wang S, You C, Chen F, Tocher DR, Li Y (2015) Characteristics of LC-PUFA  
614 biosynthesis in marine herbivorous teleost *Siganus canaliculatus* under different ambient  
615 salinities. *Aquacult Nutr* 21(5):541-551
- 616 33. Zhang Q, Xie D, Wang S, You C, Monroig O, Tocher DR, Li Y (2014) miR-17 is involved in  
617 the regulation of LC-PUFA biosynthesis in vertebrates: effects on liver expression of a fatty  
618 acyl desaturase in the marine teleost *Siganus canaliculatus*. *Biochim Biophys Acta*  
619 1841(7):934-943
- 620 34. Archer A, Lauter G, Hauptmann G, Mode A, Gustafsson J (2008) Transcriptional activity and  
621 developmental expression of liver X receptor (*lxr*) in Zebrafish. *Dev Dynam* 237(4):1090-1098
- 622 35. Cruz-Garcia L, Sanchez-Gurmaches J, Gutierrez J, Navarro I (2011) Regulation of LXR by fatty  
623 acids, insulin, growth hormone and tumor necrosis factor- $\alpha$  in rainbow trout myocytes. *Comp*  
624 *Biochem Physiol A Mol Integr Physiol* 160(2):125-136
- 625 36. Cruz-Garcia L, Sanchez-Gurmaches J, Gutierrez J, Navarro I (2012) Role of LXR in trout  
626 adipocytes: target genes, hormonal regulation, adipocyte differentiation and relation to lipolysis.

- 627       Comp Biochem Physiol A Mol Integr Physiol 163(1):120-126
- 628   37. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: molecular  
629       evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum  
630       parsimony methods. Mol Biol Evol 28(10):2731-2739
- 631   38. Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time  
632       quantitative PCR and the  $2^{-\Delta\Delta CT}$  Method. Methods 25(4):402-408
- 633   39. Alberti S, Steffensen KR, Gustafsson JA (2000) Structural characterisation of the mouse nuclear  
634       oxysterol receptor genes LXR $\alpha$  and LXR $\beta$ . Gene 243(1-2):93-103
- 635   40. Zhang Y, Mangelsdorf DJ (2002) LuXuRies of lipid homeostasis: the unity of nuclear hormone  
636       receptors, transcription regulation, and cholesterol sensing. Mol Interv 2(2):78-87
- 637   41. Cruz-Garcia L, Minghetti M, Navarro I, Tocher DR (2009) Molecular cloning, tissue expression  
638       and regulation of liver X receptor (LXR) transcription factors of Atlantic salmon (*Salmo salar*)  
639       and rainbow trout (*Oncorhynchus mykiss*). Comp Biochem Physiol B Biochem Mol Biol  
640       153(1):81-88
- 641   42. Hua X, Wu J, Goldstein JL, Brown MS, Hobbs HH (1995) Structure of the human gene  
642       encoding sterol regulatory element binding protein-1 (SREBF1) and localization of SREBF1  
643       and SREBF2 to chromosomes 17p11.2 and 22q13. Genomics 25(3):667-673
- 644   43. Dong X, Xu H, Mai K, Xu W, Zhang Y, Ai Q (2015) Cloning and characterization of SREBP-1  
645       and PPAR $\alpha$  in Japanese seabass *Lateolabrax japonicus*, and their gene expressions in response  
646       to different dietary fatty acid profiles. Comp Biochem Physiol B Biochem Mol Biol 180:48-56
- 647   44. Pai JT, Guryev O, Brown MS, Goldstein JL (1998) Differential stimulation of cholesterol and  
648       unsaturated fatty acid biosynthesis in cells expressing individual nuclear sterol regulatory



649 element-binding proteins. J Biol Chem 273(40):26138-26148

650 45. Hannah VC, Ou J, Luong A, Goldstein JL, Brown MS (2001) Unsaturated fatty acids  
651 down-regulate SREBP isoforms 1a and 1c by two mechanisms in HEK-293 cells. J Biol Chem  
652 276(6):4365-4372

653 46. Pan YX, Luo Z, Zhuo MQ, Hu W, Wu K, Shi X, Xu YH (2016) Liver X Receptor (LXR) in  
654 yellow catfish *Pelteobagrus fulvidraco*: Molecular characterization, mRNA tissue expression  
655 and transcriptional regulation by insulin *in vivo* and *in vitro*. Comp Biochem Physiol B  
656 Biochem Mol Biol 191:13-19

657 47. Peet DJ, Janowski BA, Mangelsdorf DJ (1998) The LXRs: a new class of oxysterol receptors.  
658 Curr Opin Genet Dev 8(5):571-575

659 48. Laffitte BA, Joseph SB, Walczak R, Pei L, Wilpitz DC, Collins JL, Tontonoz P (2001)  
660 Autoregulation of the human liver X receptor alpha promoter. Mol Cell Biol 21(22):7558-7568

661 49. Whitney KD, Watson MA, Goodwin B, Galardi CM, Maglich JM, Wilson JG, Willson TM,  
662 Collins JL, Kliewer SA (2001) Liver X receptor (LXR) regulation of the LXR $\alpha$  gene in human  
663 macrophages. J Biol Chem 276(47):43509-43515

664 50. Kase ET, Andersen B, Nebb HI, Rustan AC, Thoresen GH (2006) 22-Hydroxycholesterols  
665 regulate lipid metabolism differently than T0901317 in human myotubes. Biochim Biophys  
666 Acta 1761(12):1515-1522

667 51. Sprecher H (2000) Metabolism of highly unsaturated n-3 and n-6 fatty acids. Biochim Biophys  
668 Acta 1486(2-3):219-231

669 52. Tocher DR (2003) Metabolism and functions of lipids and fatty acids in teleost fish. Rev Fish  
670 Sci 11(2):107-184

- 671 53. Ou J, Tu H, Shan B, Luk A, DeBose-Boyd RA, Bashmakov Y, Goldstein JL, Brown MS (2001)  
672 Unsaturated fatty acids inhibit transcription of the sterol regulatory element-binding protein-1c  
673 (SREBP-1c) gene by antagonizing ligand-dependent activation of the LXR. Proc Natl Acad Sci  
674 USA 98(11):6027-6032
- 675 54. Xu J, Teran-Garcia M, Park JH, Nakamura MT, Clarke SD (2001) Polyunsaturated fatty acids  
676 suppress hepatic sterol regulatory element-binding protein-1 expression by accelerating  
677 transcript decay. J Biol Chem 276(13):9800-9807
- 678 55. Xu J, Cho H, O'Malley S, Park JH, Clarke SD (2002) Dietary polyunsaturated fats regulate rat  
679 liver sterol regulatory element binding proteins-1 and -2 in three distinct stages and by different  
680 mechanisms. J Nutr 132(11):3333-3339
- 681 56. Geay F, Santigosa ICE, Corporeau C, Boudry P, Dreano Y, Corcos L, Bodin N, Vandeputte M,  
682 Zambonino-Infante JL, Mazurais D, Cahu CL (2010) Regulation of FADS2 expression and  
683 activity in European sea bass (*Dicentrarchus labrax*, L.) fed a vegetable diet. Comp Biochem  
684 Physiol B Biochem Mol Biol 156(4):237-243
- 685 57. Colin S, Bourguignon E, Boullay AB, Tousaint JJ, Huet S, Caira F, Staels B, Lestavel S,  
686 Lobaccaro JM, Delerive P (2008) Intestine-specific regulation of PPAR $\alpha$  gene transcription by  
687 liver X receptors. Endocrinology 149(10):5128-5135
- 688 58. Seo JB, Moon HM, Kim WS, Lee YS, Jeong HW, Yoo EJ, Ham J, Kang H, Park MG,  
689 Steffensen KR, Stulnig TM, Gustafsson JA, Park SD, Kim JB (2004) Activated liver X  
690 receptors stimulate adipocyte differentiation through induction of peroxisome  
691 proliferator-activated receptor  $\gamma$  expression. Mol Cell Biol 24(8):3430-3444
- 692

693 **Figure legends**

694 **Fig. 1** Alignment of the deduced amino acid sequence of rabbitfish *S. canaliculatus* Lxr **(a)** and  
695 N-terminus of Srebp1 **(b)** with their orthologs in human (*H. sapiens*), mouse (*M. musculus*),  
696 chicken (*G. gallus*), zebrafish (*D. rerio*) and Atlantic salmon (*S. salar*). Identical amino acids (aa)  
697 are shaded black and similar aa are shaded grey. **(a)** Protein structural domains of Lxr are marked  
698 under the arrow line: AF-1 indicates N-terminal ligand-independent activation function (AF)  
699 domain; DBD for DNA binding domain; D region for peptides linking LBD and DBD; LBD for  
700 ligand binding domain containing the AF-2 region that is framed out in grey. **(b)** Exon regions of  
701 Srebp proteins are indicated by arrow lines.

702

703 **Fig. 2** Phylogenetic analysis of Lxr and Srebp. The deduced amino acid sequences of Lxr **(a)** and  
704 Srebp **(b)** proteins from rabbitfish and other organisms are used to construct the phylogenetic tree  
705 by the neighbor joining method with Mega5. The horizontal branch length is proportional to aa  
706 substitution rate per site. The numbers represent the frequencies with which the tree topology  
707 presented was replicated after 1000 iterations.

708

709 **Fig. 3** Tissue distribution of *Lxr* and *Srebp1* mRNA in *S. canaliculatus* examined by  
710 semi-quantitative PCR. H, heart; L, liver; S, spleen; G, gill; M, muscle; E, eyes; I, intestine; B,  
711 brain; NTC: non-template control.

712

713 **Fig. 4** Effects of the Lxr agonists T0901317, GW3965 and 22(R)-hydroxycholesterol (22HC) on the  
714 expression of *Lxr* and *Srebp1* in rabbitfish primary hepatocytes. The mRNA level of *Lxr* **(a)** and

715 *Srebp1* (**b**) were determined by qPCR in rabbitfish primary hepatocytes incubated with T0901317  
716 (1  $\mu$ M and 2  $\mu$ M), GW3965 (2  $\mu$ M and 4  $\mu$ M), 22HC (5  $\mu$ M and 10  $\mu$ M) and vehicle reagents  
717 (DMSO for T0901317, ethanol (EtOH) for 22HC) for 6 h, using *18S rRNA* as a reference gene.  
718 Data are presented as the fold change from untreated cell control in means  $\pm$  SEM (n = 3). Different  
719 superscripts above bars denote significant differences among doses ( $P < 0.05$ ; ANOVA, Tukey's  
720 test).

721

722 **Fig. 5** Effects of the Lxr agonist T0901317 on the expression of genes related to LC-PUFA  
723 biosynthesis in rabbitfish primary hepatocytes. The mRNA level of  $\Delta 4$  *Fad*,  $\Delta 6/\Delta 5$  *Fad*, *Elovl5* and  
724 *Ppar* were determined by qPCR in rabbitfish primary hepatocytes incubated with T0901317 (1  $\mu$ M  
725 and 2  $\mu$ M) or vehicle DMSO for 6 h, using *18S rRNA* as a reference gene. Data are presented as the  
726 fold change from untreated cell control in means  $\pm$  SEM (n = 3). Different superscripts above bars  
727 denote significant differences among doses ( $P < 0.05$ ; ANOVA, Tukey's test).

728

729 **Fig. 6** Expression of *Lxr* and *Srebp1* in the liver of rabbitfish fed VO and FO diets at 32 ppt and 10  
730 ppt salinity. The mRNA level of *Lxr* and *Srebp1* was determined by qPCR, using *18S rRNA* as a  
731 reference gene. Data are presented as fold change relative to initial control samples in means  $\pm$   
732 SEM (n = 6). Two-way ANOVA results were presented in the upper right corner of the figure and  
733 significant levels were considered at  $P < 0.05$ . D, diet (FO, VO); S, salinity (10, 32 ppt); D  $\times$  S,  
734 interaction; n.s., not significant.

735

736

Fig. 1

(A)

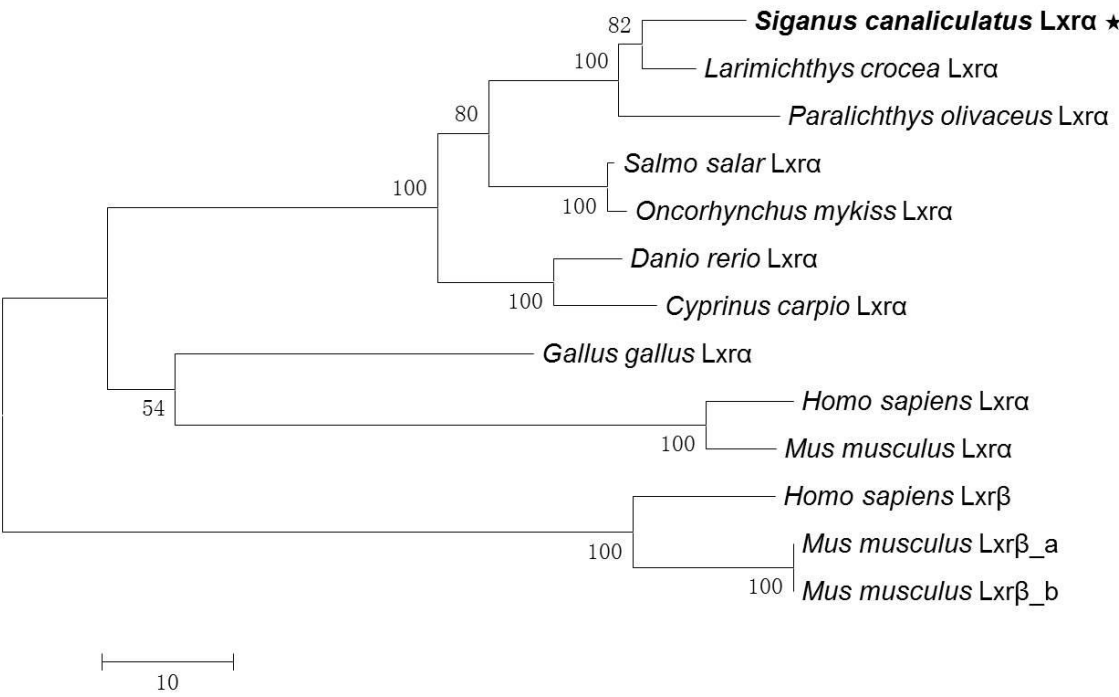
<i>H.sapiens</i>	MSLWLGAPVPDI PPDS. . . . . AVELWKP. . . . . GAQDASS QAQGS. . . . . S	37
<i>M.musculus</i>	MSLWLEASMPDVSPDS. . . . . ATELWKT. . . . . EPQDAG. . . . . DQGGN. . . . . T	35
<i>G.gallus</i>	. . . . .	0
<i>D.rerio</i>	. . . . . MAEVKQE. . . . .	7
<i>S.salar</i>	MSTLSATDI TDVGPGE. . . . . VFDGAPELQLDCLCSGDDRS SAENKHEGNLLPLEPPDHAG	56
★ <i>S.canaliculatus</i>	MSTLSVTDI SDVGHGKDETKVFDGASELQLDCMI E. ENSGSGNKKHDG. . . . . LLSLAD	53
<i>H.sapiens</i>	CI LREEARMPHS AGGTAGVGLAEAEPTALLTRAEPSPSEPTETI RPQKRKKGPAPKMLGNEL	97
<i>M.musculus</i>	CI LREEARMQSTGVALGI GLESAEPTALLPRAETLPEPTELRPQKRKKGPAPKMLGNEL	95
<i>G.gallus</i>	. MGPTQLSTQDHGKRVASVFEMEEGLSLFSGSENPPKHAENPDLKRKKGPAPKMLGNEV	59
<i>D.rerio</i>	I LSQTDL. YNTSHDDLNDMLMESNDI KMFFREDK. . . . . AAPEGQPVKRKKGPAPKMLGNEV	64
<i>S.salar</i>	FSSPPQK. GPSLSAEKSSPLNPEPSDI KADPADSMP. ANTEGQPVKRKKGPAPKMLGNEV	114
★ <i>S.canaliculatus</i>	LSHPGDFTLPHNGPSLS SPLPI EPSEVKADPAAGESSASADS QPVKRKKGPAPKMLGNEV	113
DBD		
<i>H.sapiens</i>	CSVCGDKASGFHYNVLSCEGCKGFFRRSVI KGAFYI CHSGGHCPMDTYNRRKQECRLRK	157
<i>M.musculus</i>	CSVCGDKASGFHYNVLSCEGCKGFFRRSVI KGARYVCHSGGHCPMDTYNRRKQECRLRK	155
<i>G.gallus</i>	CSVCGDKASGFHYNVLSCEGCKGFFRRSVI KGACQYVCKNGGKCEMDMYNRRKQECRLRK	119
<i>D.rerio</i>	CSVCGDKASGFHYNVLSCEGCKGFFRRSVI KGACQYSCKNSGRCEMDMYNRRKQECRLRK	124
<i>S.salar</i>	CSVCGDKASGFHYNVLSCEGCKGFFRRSVI KSAQYSCKNNGRCEMDMYNRRKQECRLRK	174
★ <i>S.canaliculatus</i>	CSVCGDKASGFHYNVLSCEGCKGFFRRSVI KSAQYSCKNNGHCEMDMYNRRKQECRLRK	173
DBD		
<i>H.sapiens</i>	CRQAGMREECVLSEEQI RLKKLKRQ. EEEQAHSATSLPPRRSSPPQI LPQLSPEQLGMI EK	216
<i>M.musculus</i>	CRQAGMREECVLSEEQI RLKKLKRQ. EEEQAQATSVS PRVSSPPQVLPQLSPEQLGMI EK	214
<i>G.gallus</i>	CQEAGMREECVLSEEQI RLKKLKRQ. EDDQARTVVVRPNPPQPPSPSHQLTPEQLNMI EK	178
<i>D.rerio</i>	CREAGMLEQCVLSEEQI RLKKMKKQ. EETARTSTVAT. . . . . PSPAPEMPLAPEQQEMI EK	181
<i>S.salar</i>	CREAGMLEQCVLSEEQI RVKKMKKN. EETARTSAVVT. . . . . PTPVPEVVPLAPEQLEMI EK	231
★ <i>S.canaliculatus</i>	CREAGMLEQCVLSEEQI RLKKMKKQHEETARTSTEST. . . . . PTFLEAAASLDPOQQEMI EK	231
DBD		
<i>H.sapiens</i>	LVAACQQCNRRSFS DRLRVTPWMPADPHS REARQQRFAHFTELAI VSVQEI VDFAKQLP	276
<i>M.musculus</i>	LVAACQQCNRRSFS DRLRVTPWPI APDQPSREARQQRFAHFTELAI VSVQEI VDFAKQLP	274
<i>G.gallus</i>	LVAACQQCNQRSFT DRLKVTWPVQVPD PNNREARQQRFAHFTELAI I VSVQEI VDFAKQLP	238
<i>D.rerio</i>	LVAACQQCNKRSFI DRPKVTPWPQS QDPQNREVRQQRFAHFTELAI MSVQEI VDFAKQLP	241
<i>S.salar</i>	LVAACQQCNKRSFI DRPKVTPWPQS QDPQNREVRQQRFAHFTELAI MSVQEI VDFAKQLP	291
★ <i>S.canaliculatus</i>	LVAACQQCNKRSFI DRPKVTPWPQS QDLQNREVRQQRFAHFTELAI MSVQEI VDFAKQLP	291
LBD		
<i>H.sapiens</i>	GFLQLSREDQI ALLKTSAI EVMLLETSRRYNPGSESI TFLKDFSYNREDFAKAGLQVEFI	336
<i>M.musculus</i>	GFLQLSREDQI ALLKTSAI EVMLLETSRRYNPGSESI TFLKDFSYNREDFAKAGLQVEFI	334
<i>G.gallus</i>	GFRELTREDQI ALLKTSI EVMLLETSRRYNPEI ESI TFLKDL SYNREDFAKAGLQFEFI	298
<i>D.rerio</i>	GFLELTREDQI ALLKTSI EI MLLETSRRYNPAI DSI TFLKDF TYNKEDFAKAGLQLEFI	301
<i>S.salar</i>	GFLELTREDQI ALLKTSI EI MLLETSRRYNPAI ESI TFLKDFSYNKEDFAKAGLQLEFI	351
★ <i>S.canaliculatus</i>	GFLELTREDQI ALLKTSI EI MLLETSRRYNPAI DSI TFLKDFSYNKEDFAKAGLQLEFI	351
LBD		
<i>H.sapiens</i>	NPI FEFSRAVNELQLNDAEFALLI AI SI FSADRPNVQDQLQVERLQHTYVEALHAYVSI H	396
<i>M.musculus</i>	NPI FEFSRAVNELQLNDAEFALLI AI SI FSADRPNVQDQLQVERLQHTYVEALHAYVSI N	394
<i>G.gallus</i>	NPI FEFSKGVNELQLNDAEYALLI AI NI FSADRPNVQDQSLVERLQHTYVEALHSYI CI N	358
<i>D.rerio</i>	NPI FEFSKGVNDLHLDAAEYALLI AI NI FSADRPNVQDHELVERLQPPYVDALHSYI RI K	361
<i>S.salar</i>	NPI FEFSKGVNDLHLDAAEYALLI AI NI FSADRPNVQDHELVERLQPPYVDALRSYI MI K	411
★ <i>S.canaliculatus</i>	NPI FEFSKGVNDLHLDAAEYALLI AI NI FSADRPNVQDHDLVERLQPPYVDALRSYI MI K	411
LBD		
<i>H.sapiens</i>	HPHDRLMFPRMLMKLVSLRTLSSVHSEQVVALRLQDKKLPPLLSEI VDVHE	447
<i>M.musculus</i>	HPHDRLMFPRMLMKLVSLRTLSSVHSEQVVALRLQDKKLPPLLSEI VDVHE	445
<i>G.gallus</i>	RPNDRLMFPRMLMKLVSLRTLSSVHSEQVVALRLQDKKLPPLLSEI VDVHE	409
<i>D.rerio</i>	RPNDRLMFPRMLMKLVSLRTLSSVHSEQVVALRLQDKKLPPLLSEI VDVHE	412
<i>S.salar</i>	RPNDRLMFPRMLMKLVSLRTLSSVHSEQVVALRLQDKKLPPLLSEI VDVHE	462
★ <i>S.canaliculatus</i>	RPNDRLMFPRMLMKLVSLRTLSSVHSEQVVALRLQDKKLPPLLSEI VDVHE	462
LBD		

(B)

<i>H.sapiens</i> Srebp1a	MDEPPFSEAAIE.....ÇAIGFECCLLAAILTTIEDMLQIINNQDSFPGFLDPE..YAG	53
<i>M.musculus</i> Srebp1a	MDELAFGEAAIE.....ÇTIAEMCELTAVLNCIEDMLQIINNQDSFPGFLDAF..YAG	53
<i>G.gallus</i> Srebp1	MSALGFDCAAIEGLA..IETRAASLIITALLSCIDDMLQIINTPDNCFSGFLDAF..FAA	56
<i>D.rerio</i> Srebp1	MN.LSFDECTSLDTLSSSIHCFSCITALLNCIIDDMLQIINTQDMFEG.LFDQASFPAP	58
<i>S.Salar</i> Srebp1	MN.LSFDEQSLDNLFETISINCFSCITALLNCIIDDMLQIINNQDMMALEFDPEQFTGV	59
★ <i>S.canaliculatus</i> Srebp1	MNSLAFDEPSLDNLFETLSINCFSCITALLSCIDDMLQIINNQDMFEPGLEFDNEPYTGT	60
<i>H.sapiens</i> Srebp1c	.....MDCTFEEDMLQIINNQDSFPGFLDAF..YAG	29
<i>M.musculus</i> Srebp1c	.....MDCTFEEDMLQIINNQDSFPGFLDPE..YAG	29

Fig. 2

(A)



(B)

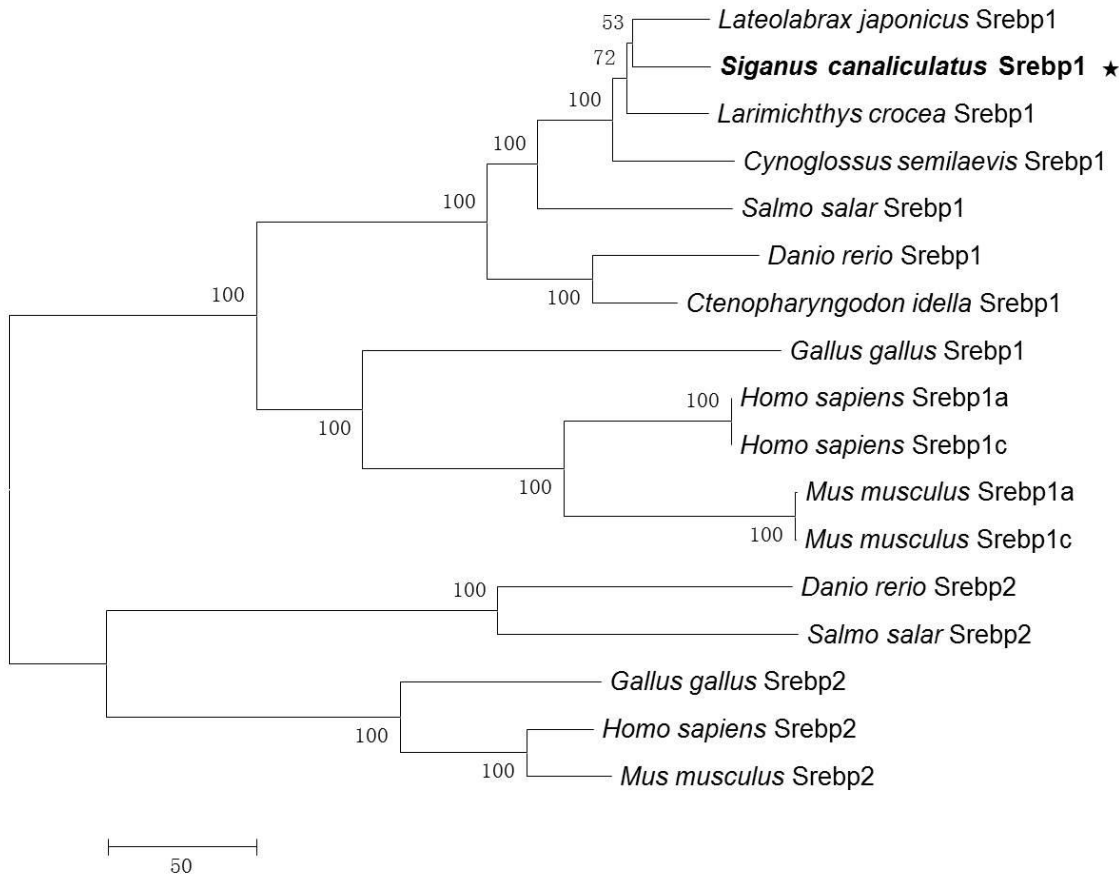
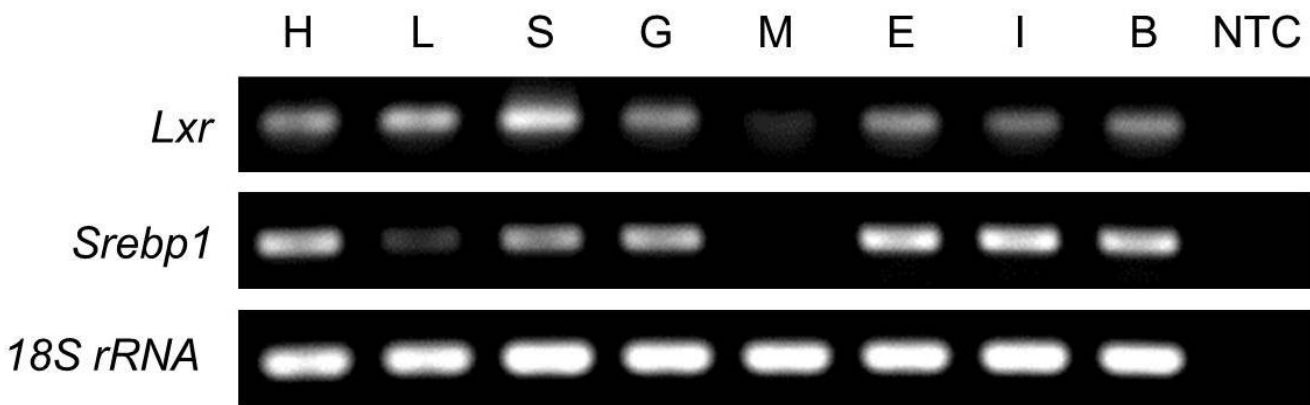
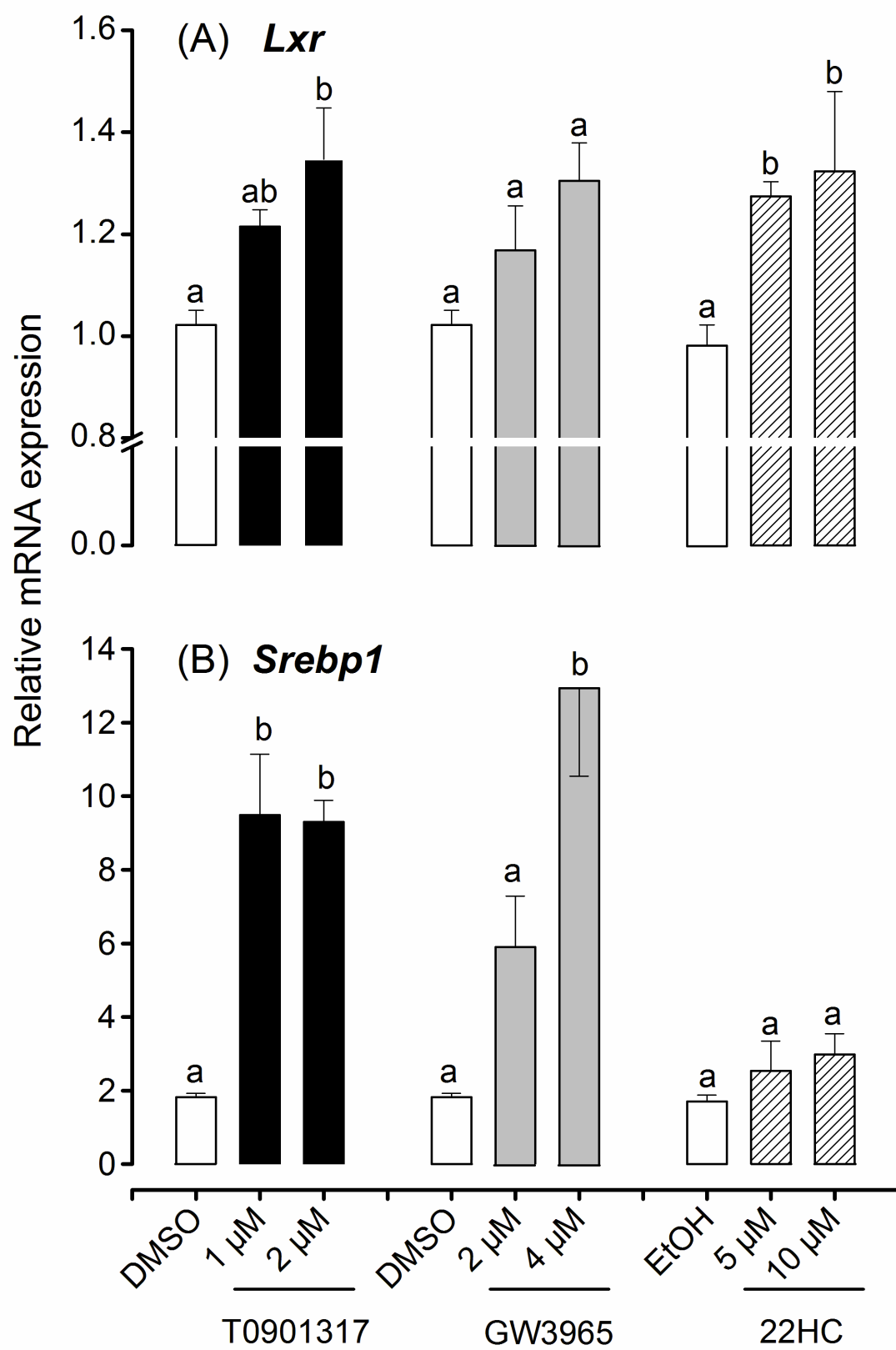


Fig. 3

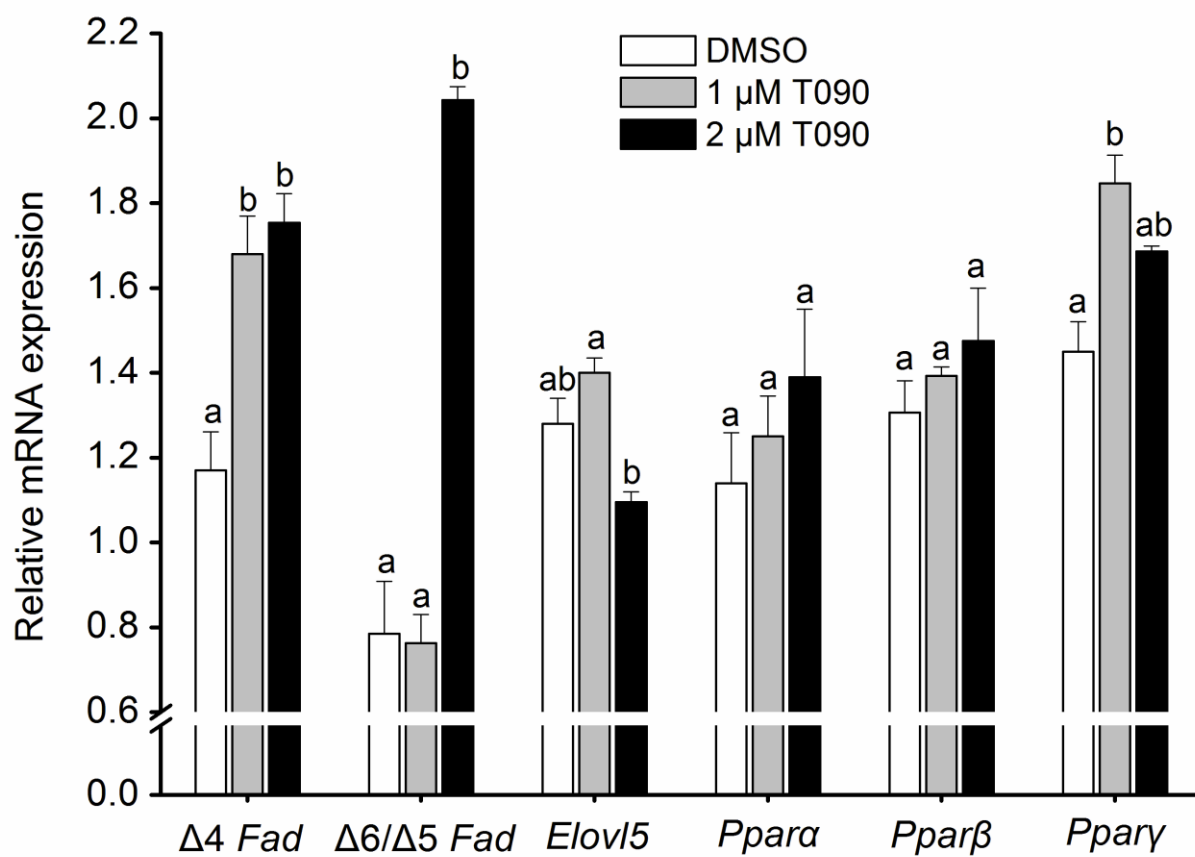




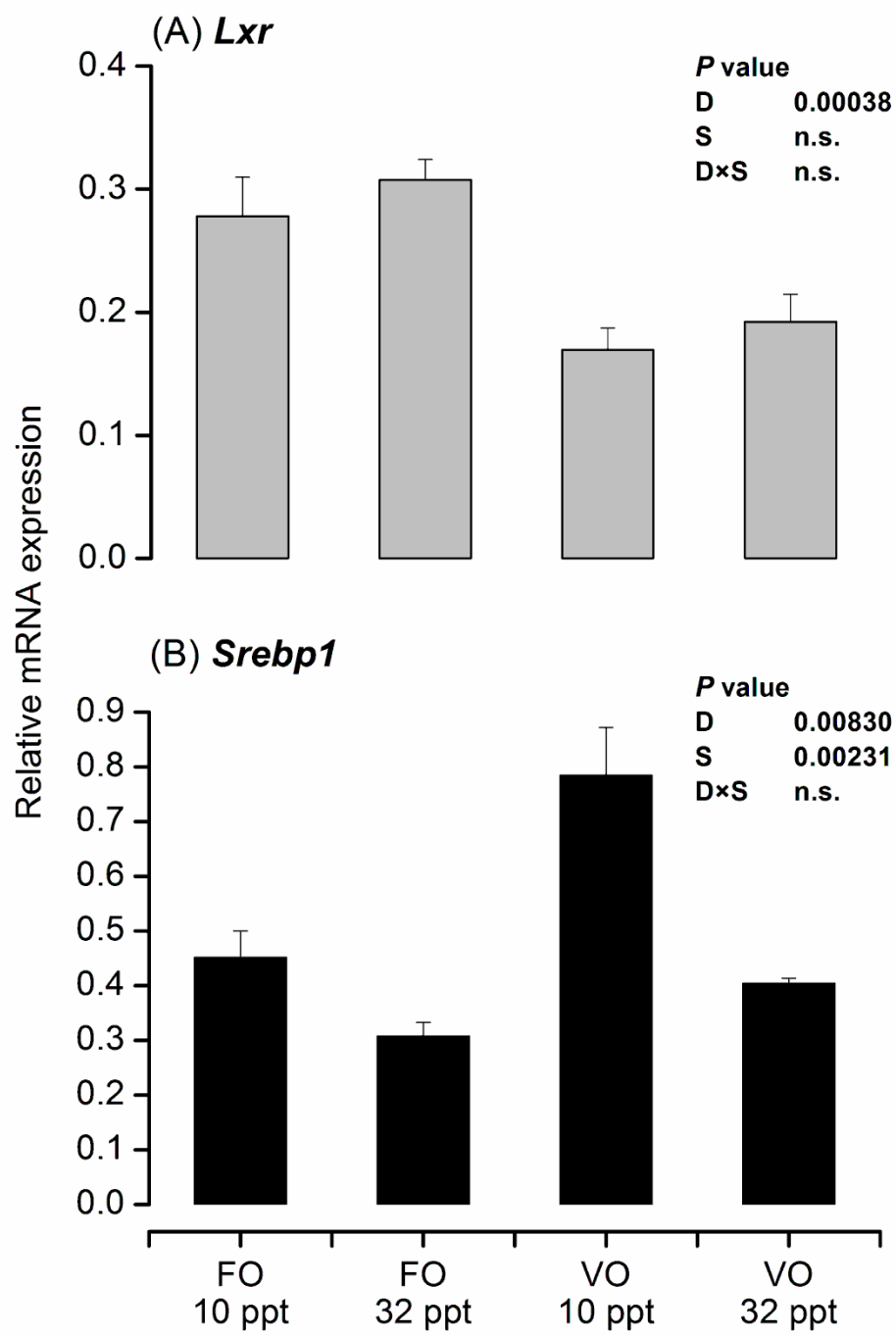
**Fig. 4**



**Fig. 5**



**Fig. 6**



**Table 1** Sequences of primers used for cloning rabbitfish *Lxr* and *Srebp1* cDNAs and for semi-quantitative PCR or qPCR.

Aim	Transcript	Forward primer	Primer sequence (5'-3')	Reverse primer	Primer sequence (5'-3')
RT-PCR	<i>Lxr</i>	lxr-partial-F	TYCAYTACAAYGTGCTGAGCTG	lxr-partial-R	CATBAGCATSCGDGGAACAT
	<i>Srebp1</i>	srebp1-partial-F	GAGCAAGTCTCTGAAGGATCTGGT	srebp1-partial-R	CCTCATCCACAAAAGAGCGGTG
RACE PCR	<i>Lxr</i>	lxr-5'-F1	CAGTGGCCGTGTTTTCAGAGGAGTAC	lxr-3'-R1	GCAGATCGACCAAAATGTCAGGATC
					A
		lxr-5'-F2	GCGTAAGCGGCACTGCTGACATTTC	lxr-3'-R2	GTCTCGGGGTACAATCCTGCTATCG
	<i>Srebp1</i>	srebp1-5'-F1	TGGCTGTGACCCCTCACTCCAAAGT	srebp1-5'-R1	CATGGGACTATCCGGTTCGGAATC
		srebp1-5'-F2	AGAGTCTGTGCCCCAAGGCGTGT	srebp1-5'-R2	TGGGCAGCTCACTCTTCACATCAG
Full length clone	<i>Lxr</i>	lxr-full-F	AGGGCAACTGTGCTCTCATCC	lxr-full-R	TTGGCAGTCTGAGGTCATGGAC
	<i>Srebp1</i>	srebp1-full-F	GCGGTTTCAGGTACACTGGATT	srebp1-full-R	CCCTTCTGCCTAGCTGTTGGT
qPCR <sup>a</sup>	<i>Lxr</i>	lxr-qPCR-F	CTTTGCCCTTCGCCCTCCAG	lxr-qPCR-R	TGTTCTGCCAGACGCCAC
	<i>Srebp1</i>	srebp1-qPCR-F	CGGAGCCAAAGACAGAGGAGTG	srebp1-qPCR-R	GTCTCCCAGCTTCTCCAAGGTAC
	$\Delta 4$ <i>Fad</i>	$\Delta 4$ fad-qPCR-F	TCACTGGAACCTGCCACACAT	$\Delta 4$ fad-qPCR-R	TTCATTCTCAGACAGTGCAAAACAG
	$\Delta 6$ <i>Fad</i>	$\Delta 6$ fad-qPCR-F	TGCACCTAACAGGAGTCACGTT	$\Delta 6$ fad-qPCR-R	GCTCAGCACAGGATTGAGTACG
	<i>Elovl5</i>	elovl5-qPCR-F	CACAGCTTCAGCTGGTCCAG	elovl5-qPCR-R	TGCGTTTCTTATAAGTCTGAATGTAG
	<i>Ppara</i>	ppara-qPCR-F	CAAGGCTGAGAGCAAGATGGT	ppara-qPCR-R	AAGGCGTCTGCTGGTCTTTTC
	<i>Ppar<math>\beta</math></i>	ppar $\beta$ -qPCR-F	TGCTAGCAGAGGAACTGAACCTTG	ppar $\beta$ -qPCR-R	AACGGCGAGGTGCTGCTT
	<i>Ppar<math>\gamma</math></i>	ppar $\gamma$ -qPCR-F	CTGCTGGCTGAGTTCTCGTCT	ppar $\gamma$ -qPCR-R	ATGACAAAAGGCGCGTTATCTC
	18S rRNA	18S rRNA-qPCR-F	CGCCGAGAAAGACGATCAAAC	18S rRNA-qPCR-R	TGATCCTTCCGCAAGTTTAC

<sup>a</sup> The qPCR primers for *Lxr* and *Srebp1* were also used in the semi-quantitative PCR assays